1) Why has Sonic Genetics decided to add the 22q11.2 test option to its Harmony® testing menu?

It is Sonic Genetic’s desire to provide clinically relevant NIPT options for clinicians and patients. Assessment of the scientific literature and discussions with thought leaders in the field of prenatal diagnosis demonstrated the medical value of NIPT for 22q11.2 deletion. 22q11.2 deletion is the most common microdeletion syndrome, occurring in as many as 1 in 1,000 pregnancies. It is one of the most common cause of developmental delay after Down syndrome. It has a well-described, if variable phenotype, which may include congenital heart defect, immune deficiency, palate anomalies leading to feeding and speech problems, renal anomalies and psychiatric conditions.

2) What are the benefits of prenatal screening for 22q11.2 deletion?

Conventional screening methods, such as first trimester screening, do not reliably detect 22q11.2 deletion in the prenatal period. In addition, maternal age is not a risk factor for microdeletions, so 22q11.2 deletion syndrome can occur in any pregnancy.

Early prenatal screening for 22q11.2 deletion, combined with confirmatory diagnosis, is critical for appropriate medical management of the pregnancy, as well as for the child in the period immediately following delivery and into childhood. For example, if a fetus is at increased risk for 22q11.2 deletion, a specialised ultrasound of the fetal heart (fetal echocardiogram) is recommended to evaluate for congenital heart defects. Prenatal screening and early diagnosis also enable family planning and informed decision making for subsequent pregnancies.

3) Can you provide more information about the validation data for 22q11.2 deletion?

Performance claims for the 22q11.2 test are based on the analytical validation study described below. Before launch of the 22q11.2 test, Roche performed a robust validation study of 122 samples affected with 22q11.2 deletion, including samples from confirmed affected pregnancies, as well as simulated pregnancy samples, and 1,614 samples from presumed unaffected pregnancies. Singleton pregnancies, including IVF pregnancies with self and non-self donor egg, were included.

Deletion sizes in affected samples ranged from 1.96–3.25 Mb. A wide range of fetal fractions were assessed: roughly one-third of affected samples had 4-10% fetal fraction, one-third had 10-15%, and the remaining one-third had >15% fetal fraction. This study represents, to our knowledge, the largest and most comprehensive prospective validation of a cell-free DNA test for 22q11.2 deletion.

The sensitivity of the test is 75% [95% CI: 67-82%]. In other words, the 22q11.2 test is expected to yield a ‘high probability’ result in 75% of pregnancies affected with 22q11.2 deletion. The specificity of the 22q11.2 test is 99.5% [95% CI: 99.0-99.7%]. This corresponds to a ‘false-positive’ rate of 0.5% (or 1 in 200). In other words, if a pregnancy is NOT affected with 22q11.2 deletion, there is a 0.5% chance of receiving a ‘high probability’ result for the 22q11.2 test. In addition, there are ongoing efforts to analyse a larger number of samples from affected pregnancies, and enrolment is ongoing for a prospective clinical study.
Non-invasive prenatal testing for 22q11.2 deletion

Performance and accuracy

The Harmony Prenatal Test has an overall low cumulative false-positive rate. For trisomies 21, 18, 13 and 22q11.2 deletion, the cumulative false-positive rate is less than 0.6%.  

<table>
<thead>
<tr>
<th>22q11.2 deletion</th>
<th>Detection rate</th>
<th>False-positive rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>within the 3 Mb region*</td>
<td>75% (^7)</td>
<td>0.5% (^7)</td>
</tr>
</tbody>
</table>

*including smaller nested deletions

22q11.2 deletion: Microdeletion panels

4) I currently offer an NIPT microdeletion panel. Why would I want to test for only 22q11.2 and not a panel of microdeletions?

The Harmony test with 22q11.2 deletion focuses on clinically relevant conditions, minimising the chance of a false-positive result as compared to panels that cover very rare microdeletions.

Often, the conditions included on these panels are exceedingly rare (for example, Wolf-Hirschhorn syndrome (WHS) is included on some panels, which has a prevalence of 1 in 50,000 live births). \(^8\) It is important to consider that each condition tested with NIPT has an associated false-positive rate and that false-positive rates are cumulative. Therefore, each microdeletion condition tested has an associated false-positive rate and adds to the total false-positive rate of the test. As some of these conditions are so rare, ‘false-positive’ results are likely to be much more common than ‘true-positive’ results. In addition, validation data for these rare microdeletions is extremely limited and performance is poorly understood. If patients are concerned about the risk of rare microdeletions, diagnostic testing with microarray should be considered.

The Harmony test focuses on clinically relevant conditions and provides flexibility to order only the tests that are appropriate in a given situation. This minimises unnecessary invasive procedures due to false-positive results, while addressing the conditions that are likely to be of greatest concern for patients and clinicians.

5) Other NIPT laboratories claim a higher sensitivity for 22q11.2. Why should I switch to using the Harmony test?

There are many factors to consider when choosing an NIPT. Test sensitivity (or detection rate) is one of these factors. Other factors include overall test performance and quality (such as accurate measurement of fetal fraction). To date, published validation studies of 22q11.2 performance have been limited. Some validation studies have only included samples with the 3 Mb deletion which is present in 85% of patients (Panorama analytical validation study), and do not address performance of the test in patients with smaller or atypical deletions. A test that cannot detect deletions smaller than 3 Mb will, by definition, have a detection rate of less than 85% in clinical practice. \(^9\)

In contrast, Roche has performed the largest analytical validation study to date for 22q11.2 deletion \((n=1,736)\), including deletion sizes ranging from 1.96 to 3.25 Mb and fetal fraction from 4 to 33%. \(^7\)

It is important to consider that each condition tested with NIPT has an associated false-positive rate and that false-positive rates are cumulative. A lower false-positive rate may reduce patient anxiety and unnecessary invasive procedures. \(^7\) The combined false-positive rate for Harmony trisomies 21, 18 and 13, with 22q11.2 remains exceptionally low. It is less than 0.6%. \(^7,8\)
22q11.2 deletion: Clinical considerations

6) Does this test provide a probability assessment for 22q11.2 duplications?

The 22q11.2 test option assesses the probability of 22q11.2 deletion. It does not provide a probability assessment for 22q11.2 duplication.

7) My patient has a diagnosis of 22q11.2 deletion. Is she eligible for the 22q11.2 test?

No. The 22q11.2 test option cannot distinguish between maternal and fetal 22q11.2 deletions. A ‘high probability of a deletion’ result indicates that the analysis detected a decrease of cell-free DNA fragments consistent with a deletion in the 22q11.2 region, which may be fetal, maternal or both. NIPT is a screening test. If a pregnancy is known to be at increased risk for 22q11.2 deletion, based on family history or ultrasound findings, diagnostic testing should be considered.

8) Which patients are eligible for the 22q11.2 test?

The 22q11.2 test has been validated in singleton pregnancies, including IVF self and non-self egg donor pregnancies. This test has not been validated in pregnancies with more than one fetus and is not suitable for women with a known 22q11.2 deletion.

Please be aware that Harmony is a screening test, intended to assess the probability of specific conditions. Not all affected pregnancies will receive a ‘high probability’ result, and some unaffected pregnancies will receive a ‘high probability’ result.

A fetus with a congenital heart defect may be at increased risk for other microdeletion syndromes and genetic conditions not addressed by NIPT. If a pregnancy is known to be at increased risk for 22q11.2 deletion, based on ultrasound findings, diagnostic testing by microarray analysis should be considered.

22q11.2 deletion: Ordering

9) How do I request the 22q11.2 test?

If you and your patient have decided to include the 22q11.2 deletion option as an add-on to the core Harmony test, then it must be selected on the request form. If you do not have a request form that includes the 22q option, please download one from the Sonic Genetics website, www.sonicgenetics.com.au/nipt. Please note: The Harmony Prenatal Test Request Form is also available on most of the popular Practice Management Software systems.

10) Can I order the 22q11.2 test as a stand-alone test?

The 22q11.2 test is an optional add-on to the core Harmony test for trisomy 21, trisomy 18 and trisomy 13. The 22q11.2 test is not available as a stand-alone test.

11) I ordered only the core Harmony test for my patient earlier in her pregnancy. Can I add the 22q11.2 test to the patient’s previously analysed specimen?

You may be able to add the 22q11.2 test for a patient sample that has already yielded a Harmony test report or is currently being processed at the laboratory. The 22q11.2 test cannot be added to specimens that are not eligible for the test (e.g. twin pregnancy). The 22q11.2 test can be added up to two weeks after the result for the aneuploidy screen. Apart from paying the difference between the two tests, there is also a minor re-analysis fee in such instances.

12) Will the turnaround time be the same if I order the 22q11.2 test with the Harmony test?

Yes. You will experience the same rapid turnaround whether or not you order the 22q11.2 test.

13) Is a change in sample volume required to run the 22q11.2 test?

No. The 22q11.2 test option can be added to the Harmony test with no change in required sample volume.

14) What is the minimum fetal fraction required to obtain a test result for 22q11.2?

The fetal fraction cut-off for the 22q11.2 test is the same as the cut-off for the core Harmony test, that is, the fetal fraction must be greater than or equal to 4%.
22q11.2 deletion: Results

15) What does a ‘high probability of a deletion’ result indicate? What is the recommended follow-up?

A ‘high probability of a deletion’ result indicates that the analysis detected a decrease of cell-free DNA fragments consistent with a deletion in the 22q11.2 region. As the cell-free DNA in a specimen originates from both the mother and the pregnancy, the test does not distinguish between a 22q11.2 deletion in the woman, the pregnancy, or in both the woman and the pregnancy. If a definitive diagnosis is desired prenatally, amniocentesis or CVS with FISH (fluorescent in-situ hybridisation) or microarray analysis should be considered. Sonic Genetics provides these tests for patients that fulfil the Medicare criteria and no out-of-pocket expense is incurred. Conventional karyotyping should not be used to confirm a high probability result. In addition, level II ultrasound with fetal echocardiogram is recommended to evaluate for anomalies such as congenital heart defect, cleft palate, etc., and delivery at a tertiary care centre should be considered, if possible. 8

16) What does a ‘no evidence of a deletion’ result indicate? Does it exclude a 22q11.2 deletion in the fetus?

‘No evidence of a deletion observed’ indicates the analysis did not find an increased probability for a deletion in the 22q11.2 region. Harmony is a screening test. Not all fetuses with 22q11.2 deletions will be classified as ‘high probability’. This test does not rule out the possibility of other clinically significant aneuploidy, single gene conditions, microdeletions, or microduplications being present in the fetus. There are both biological and technical reasons as to why NIPT by cell-free DNA cannot provide a definitive diagnosis, regardless of test type or laboratory. Results should be interpreted in the context of the complete clinical history, including ultrasound findings and family history.

17) What is the positive predictive value (PPV) of the 22q11.2 test?

The positive predictive value of any test depends on the prevalence of the condition in the population tested and the ‘false-positive’ rate of the test (PPV = true-positives/false-positives + true-positives). Due to the fact that not all cases of 22q11.2 deletion are diagnosed in either the prenatal or the postnatal period, the prevalence of this condition is not well defined. Theoretical PPV calculations make significant assumptions and their output depends on the accuracy of the information used in the calculation. Clinicians should exercise caution when using these calculations. They are intended for educational purposes and not for direct clinical application.

Benefits of the Harmony Prenatal Test

- Flexible testing options and clinically relevant testing
- Reliable timely results regardless of test options ordered
- Minimise unnecessary invasive procedures due to false-positives
- Single blood collection regardless of test options ordered

Performed in Australia in our NATA-accredited Sullivan Nicolaides Pathology laboratory

For further information, including scientific and peer-reviewed publications, please refer to our website, www.sonicgenetics.com.au/nipt or call us on 1800 010 447

References


For further information, including scientific and peer-reviewed publications, please refer to our website, www.sonicgenetics.com.au/nipt or call us on 1800 010 447

© 2018 Roche Diagnostics. 

Non-invasive prenatal testing based on cell-free DNA analysis is not diagnostic: results should be confirmed by diagnostic testing. Before making any treatment decisions, all women should discuss their results with their healthcare provider, who can recommend confirmatory, diagnostic testing where appropriate. The Harmony Prenatal Test was developed by Ariosa Diagnostics. Sonic Genetics performs the Harmony Prenatal Test in Australia at our NATA-accredited Sullivan Nicolaides Pathology (SNP) laboratory. The Harmony Prenatal Test is included on the Australian Register of Therapeutic Goods.

SHG-MKT-0027-00. 1

1800 010 447 | E info@sonicgenetics.com.au
www.sonicgenetics.com.au

Printed June 2018